Using Molecular Modeling to Predict Spectral Characteristics of Peroxidase Products

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Introduction

Determining spectral changes when reactants are converted to products is a convenient and sensitive method of monitoring the progress of reactions. It is useful to know ahead of time the spectral characteristics of the anticipated products. This is not always possible as the products may not be commercially available and/or spectral information is unknown. Molecular modeling provides a potential means of predicting the spectral characteristics of compounds that may be formed during a reaction (in some cases multiple products might actually be formed). To test this hypothesis, CAChe, a molecular modeling program, was used to predict the spectral characteristics of ethyl ferulate, typical ferulate dehydrodimers, sinapyl alcohol, and potential cross reaction products of sinapyl alcohol. Authentic samples of ethyl ferulate and sinapyl alcohol were available for verification of the predicted electronic spectra.

Materials and Methods

The CAChe system from Oxford Molecular Group was used to build models of predicted structures of dimers formed from sinapyl alcohol and ethyl ferulate. Molecular structures were optimized using MM2 molecular mechanic parameters, and ZINDO (Zerner's Intermediate Neglect of Differential Overlap) program was used to compute spectroscopic properties (electronic spectra) of the molecules.

Results and Discussion

Ethyl ferulate dehydrodimers, 8-5 (1), 8-*O*-4 (2), 5-5 (3), and 8-8 (4) (Fig. 1), were built using the CAChe molecular modeling program and all structures optimized for lowest energy using the

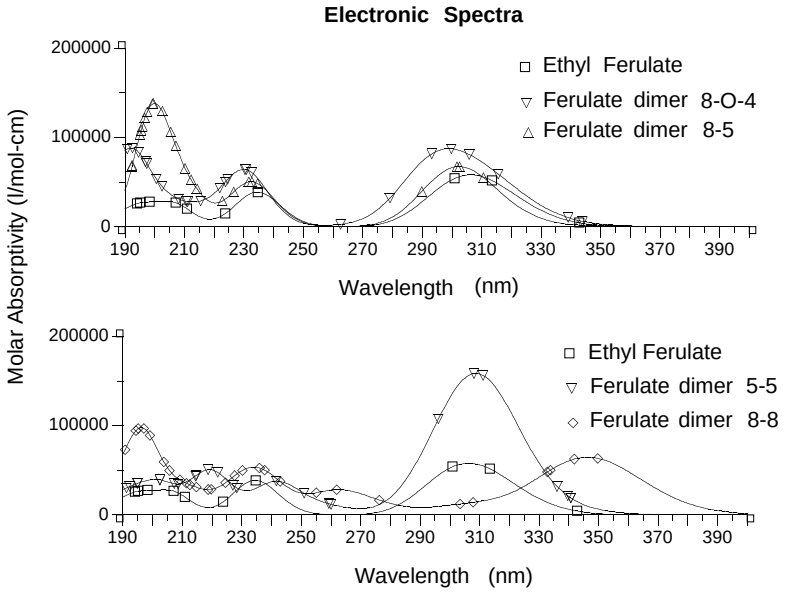

Figure 1. Ferulate dehydrodimers analyzed for spectral characteristics using the CAChe Molecular Modeling Program. Coupling products of sinapyl alcohol modeled with CAChe.

MM2 parameters. Predicted electronic spectra for the compounds are given in Fig 2. Although these predictions were based on molecules in a null environment having no other chemical interactions, the predicted maximum at 300 nm (Fig. 2) was similar to that observed for ethyl ferulate in an organic solvent such as methanol (maximum at 310 nm). The predicted spectra for the ferulate dimers show slight changes in the maximum around 300 nm except for the 8-8 dimer that has a significant shift to a longer wavelength (345 nm). These predicted values are consistent with observed spectra of ethyl ferulate dimers. Although the spectral region from 190 to 240 would be much more diagnostic for the ferulate dimers, this region is of little practical use in monitoring cross coupling reactions mediated by peroxidases due to the strong absorbance displayed by most buffering systems in this region.

Electronic spectral predictions for sinapyl alcohol and two potential cross-coupling products are shown in Fig. 3. In both cases the dimers have significantly different spectral properties from the sinapyl alcohol. We have not been able to obtain actual spectra for the two dimer products, but the predicted spectra for sinapyl alcohol is close to the observed spectra (maximum at 270 nm). From monitoring peroxidase mediated coupling reactions of sinapyl alcohol, there is a significant loss of the molar absorptivity maximum at 270 nm. Again the region from 190 to 230 nm would be diagnostic but is unavailable due to interference from buffers and solvents used to obtain the electronic spectra.

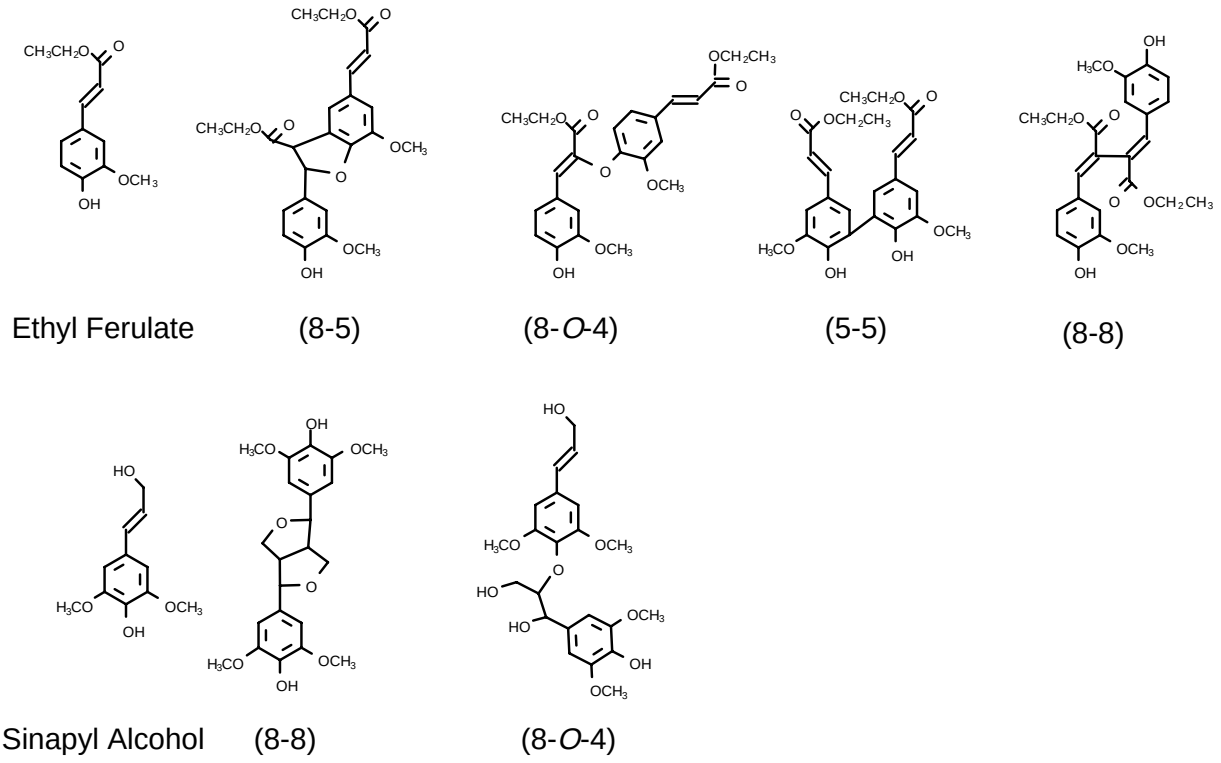

Figure 2. Electronic spectra predicted for ferulate dimers.

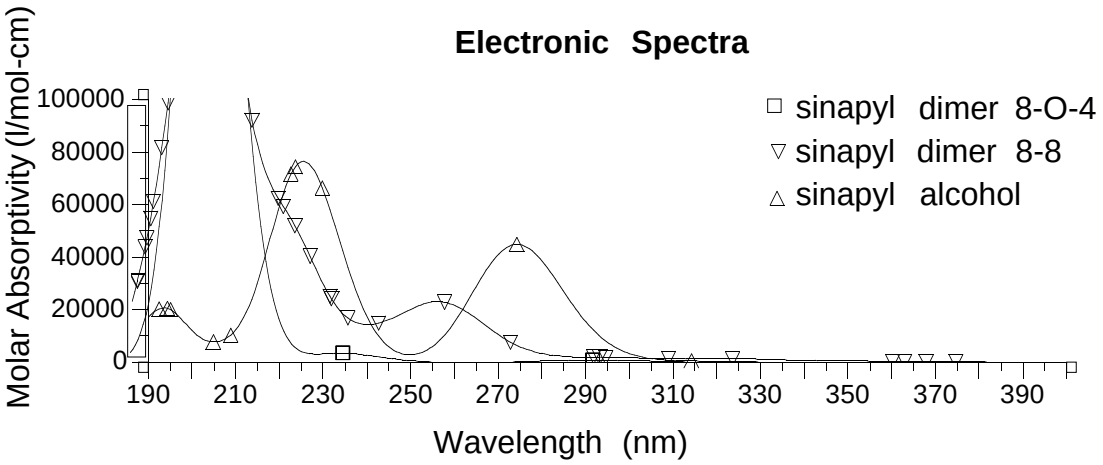

Figure 3. Electronic spectra predicted for sinapyl alcohol dimers.

Conclusion

Although it is not possible with this program to predict the precise electronic spectra, due to solvent influences, the predictions in a null environment are close to observed values. This is a useful tool to predict electronic spectra of small molecules providing insight into possible changes during certain types of reactions.

Impact

Development of spectral assays for monitoring reactions allows us greater flexibility in characterizing enzyme properties on small amounts of material. As our knowledge base increases, we are better able to understand and predict metabolic changes in plant cell wall chemistry/biochemistry leading to potential methods for improving forage utilization.